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The Effect of Pangolagrass, *Digitaria Decumbens*
Stent, on the Cotton Root-Knot Nematode,
Meloidogyne incognita acrita Chitwood

By
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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

June, 1962

ACKNOWLEDGMENTS

The author wishes to express deep appreciation to Dr. V. G. Perry for enthusiastic guidance and instruction in Nematology and in the supervision of this research. He is also deeply indebted to Mr. N. C. Hayslip for encouragement and cooperation in this work.

He wishes to express his appreciation to Doctors W. T. Forsee, Jr., E. G. Rodgers, G. D. Thornton, and A. A. DiEdwardo, the members of his Graduate Committee, and to Dr. J. T. Creighton, for their supervision of his graduate program and for their many helpful criticisms and suggestions offered in the preparation of this dissertation.

He also wishes to express his appreciation to the University of Florida Agricultural Experiment Station for permitting the use of data he collected under Project 712 in this dissertation.

Finally, to his wife and sons, he wishes to convey his appreciation for their understanding and cooperation during this period of graduate study.

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INTRODUCTION

The several species that make up the nematode genus Meloidogyne incite important diseases of many cultivated and wild plants. These diseases are collectively referred to as root-knot, and the nematodes are commonly called root-knot nematodes. Known hosts approach 2,000 species of plants, but the typical symptoms of galling are lacking when many hosts, especially the grasses, are parasitized. These nematodes have been reported from most areas of the United States. Some are particularly abundant in the sandy soils of the South where climatic conditions are favorable for their growth and development.

Crops used in rotations to control root-knot nematodes have, in the past, been selected because in some way or another they decreased populations of the parasites. Apparently, the parasites do not penetrate the roots of these plants; or, if penetration is accomplished, adequate nutrition for reproduction is not provided by the host.

Nematodes respond in a variety of ways to exudates or secretions from plants. The most important of these

responses is probably the attraction of the nematode to plant roots by exudates which activate the chemoreceptor systems of nematodes. In at least a few cases, it has been definitely established that materials exuded by plant roots stimulate hatching of nematode eggs in some manner. These two types of response would thus be largely beneficial to the nematodes. On the other hand, certain plants apparently produce and release into the soil materials that are toxic to the nematodes. Even the so-called "hatching-factors" may be detrimental to individual species when suitable host tissue is not present. Thus it seems possible that plants which produce such material might provide a more effective means of reducing nematode populations in the soil than do those plants which merely fail to provide food for completion of the nematode life cycle.

This investigation was initiated to determine if any of the commonly grown pasture and crop plants of South Florida could be used in rotation with tomatoes and produce control of the cotton root-knot nematode, Meloidogyne incognita acrita Chitwood, 1949. Particular attention was directed to the possible effects that root exudates of pangolagrass, Digitaria decumbens Stent, might have on this nematode.

CHAPTER I

LITERATURE REVIEW

Root-knot was first reported in 1855 by Berkeley (4) who described the symptoms caused by this nematode on cucumbers. In separate monographs by Atkinson (1) and Neal (32) in 1889, the disease was described on a number of plants from Alabama and Florida. Neal stated that it had been recognized as an important disease of Florida crops as early as 1805 and suggested the cultivation of resistant plants in rotation with susceptible crops as a means of control.

Previous to a study by Chitwood (9) in 1949, all root-knot nematodes were considered as a single species belonging to the genus Heterodera. Christie and Albin (12) in 1944 demonstrated physiological differences between different populations of the nematode. Chitwood (9) initiated a study which led to placing all root-knot nematodes in the genus Meloidogyne Goldi, 1887. He was able to distinguish four species and two closely related sub-species, M. incognita incognita and M. incognita acrita. Although

these appear to differ in both morphology and physiology, Triantaphyllou and Sasser (50) proposed that they be considered synonymous. However, the two sub-species appear heterogenous in that populations collected from different localities react differently under the same environment. The nematode populations used in this study were definitely identified as M. incognita acrita according to descriptions of the nematodes by Chitwood (9) and Taylor et al. (48).

Numerous workers have investigated crop rotation for root-knot control with varying success. Finding several resistant crops which can be used in a crop rotation program is often difficult because of the wide host range of this nematode. Steiner (45) observed that plants widely separated taxonomically tended to decrease nematode populations even though they were known hosts. He made the interesting suggestion that two or more crops known to be suitable hosts for these nematodes might be successfully used in rotations. Garris (21) suggested varying or "rotating" the rotation for even greater nematode control.

The forms of host resistance to plant parasitic nematodes, as well as concepts of a resistant plant, vary greatly. Tyler (54) defined a root-knot resistant plant as one in which penetration does not occur, or does so to

only a limited extent. She thought the plant root tissue prevented penetration, while Sasser and Taylor (43) thought that resistance was due to a lack of ability of the larvae to enter the root. Christie (10) used the term "suitable host" for a plant on which nematodes grew and reproduced and "unsuitable host" for a plant on which they failed to grow and reproduce, or did so to a limited extent, thus avoiding the term "resistance."

One type of resistance is demonstrated by plants such as Crotalaria spectabilis Roth, Solanus grandiflorum R. and P., Lantana (Lantana camara L.), and silver cineraria (Senecio cineraria DC.), in which, according to Steiner (45), root-knot larvae freely penetrate the roots but fail to reach maturity. Another type of resistance is exhibited by the rose geranium (probably Pelargonium graveolens, L'Her.). In this plant, the basal portion of the stem seems to be attractive to nematodes and is invaded by large numbers of larvae which develop to maturity, but the roots of this plant are resistant to the nematodes and do not allow penetration. Weiser (60) found that the apical meristem of tomato root was repellent, the region of elongation attractive, and the piliferous region neutral or slightly repellent to Meloidogyne hapla Chitwood, 1949, while all parts

of another very susceptible host plant, bean (Phaseolus vulgaris, var. pinto), were repellent. Using a pure line of M. incoqnita (Kofoed and White) Chitwood, 1949, on tomato, Peacock (38) found no evidence of this repellency but did observe that the larvae were attracted to the region of root elongation. He also demonstrated the attraction more positively than any other worker by the placing of a sheet of cellophane between the larvae and the root tip. The larvae clustered opposite the growing point, and moved along with the root tip as it grew away.

Another type of resistance is demonstrated by plants which produce root diffusates which are toxic to nematodes. These plants include African marigolds (Tagetes erecta and T. patula), and asparagus, Asparagus officinalis var. altilis L. Oostenbrink et al. (36) demonstrated that African marigolds are effective in reducing populations of Pratylenchus penetrans (Cobb, 1917) Sher and Allen, 1953, and P. pratensis (de Mann, 1880) Filipjev, 1936. Oostenbrink (35) also reported that a population of M. hapla Chitwood, 1949, was reduced significantly by marigolds. He further reported that although Rotylenchus robustus (de Mann, 1876) Filipjev, 1936, appeared to be suppressed when its original population was high, a moderate population

maintained itself as long as four years under continuous culture of marigolds. Populations of P. coffeae (Zimmerman, 1898) Goodey, 1951, and M. javanica (Treub, 1885) Chitwood, 1949, in soils used for tea production were considerably reduced by cultivation of T. erecta and T. patula, according to Visser and Vythilingam (59). They suggested the use of these plants as a cover crop in young tea, but not in mature tea, because the cultural practices essential for tea would be detrimental to marigold production.

Uhlenbroek and Bijloo (55, 56) found that ethanol extracts of T. nana plants showed a moderately high nematocidal activity against several plant parasitic nematodes. They were able to extract and identify three compounds with nematocidal properties from this plant.

Asparagus was shown by Rohde and Jenkins (41) to be toxic to stubby-root nematode, Trichodorus christiei Allen, with the nematocidal effect of the plant increasing as the fleshy storage roots were formed. They theorized that a compound (probably a glycoside) toxic to nematodes was present in the rhizosphere of asparagus, causing direct kill or disruption of the reproductive processes. Solutions of the toxic material, when drenched into the soil or sprayed directly on the leaves, decreased T. christiei populations on tomato.

Another response to root diffusates of certain plants is that of induced egg hatching which is well known with the members of Heterodera but only recently observed in Meloidogyne. Abundant hatching of Heterodera eggs is known to require a more or less specific external environmental alteration normally provided by a suitable host. Until such time as these conditions are provided, the eggs survive and retain their infectivity. Many investigations have shown that eggs of Heterodera rostochiensis Wollenweber, 1923, hatch abundantly when exposed to a so-called "hatching factor" exuded from the roots of potato or tomato. In the absence of the "hatching factors" few eggs will hatch. According to Winslow (67), H. rostochiensis displays a greater degree of host specificity than most other plant parasitic nematodes. Baunacke (2) demonstrated that exudates obtained by washing sugar beet roots increased larval emergence of the sugar beet nematode, H. schachtii Schmidt, 1871. Rensch (40) then attempted to isolate the active material and synthetically produced two compounds, A and B, which greatly increased the rate of hatching H. schachtii eggs. Thorne (49) found that H. schachtii eggs were stimulated to hatch by the sugar beet, but he observed that only about one-third of the larvae emerged from the

eggs in the presence of young sugar beet roots. He suggested that when such a large portion of the larvae fail to respond to young sugar beet roots, there must be other factors present which inhibit emergence. Ouden (37) reported that root exudates of Hesperis matronalis L., a small cruciferous weed from Holland, were as effective as those of the sugar beet in causing H. schachtii eggs to hatch. This weed is not a suitable host for H. schachtii (67), and it can remain in the soil as long as is necessary to stimulate hatching of the eggs and thus bring about control through starvation.

Several workers have investigated the effect of potato root diffusates on H. rostochiensis and other cyst nematodes. Triffitt (51) observed that potato root diffusates increased the hatching of eggs of a cyst nematode and suggested that mustard root diffusates could counteract the effect of the potato root diffusate. In later work (52, 53), she presented data showing that certain grasses produce diffusates which stimulate the hatching of eggs of some cyst nematodes. Fenwick (16) suggested that root diffusates can induce emergence of larvae from cysts and that this action is specific, i.e., a given species of nematode can be stimulated by diffusates from certain plants only. He (17) developed a hatching curve for H. rostochiensis

which could be used in forecasting final numbers of emerged larvae from a group of cysts. This information is useful because hatching of all of the eggs in a group of cysts often requires several months in the laboratory. The production, concentration, and storage of potato-diffusates has been studied by Widdowson (62, 63, 64, 65, 66), who noted that the peak of root diffusate activity occurred about four weeks after the emergence of the potato shoot.

Meloidogyne larvae emerge readily from egg masses when temperature, moisture, and aeration are favorable, according to Godfrey (22) and Bergeson (3). In tests by Viglierchio and Lownsberry (57, 58), substances produced by tomato roots stimulated the hatching of root-knot eggs. In their tests, larvae emerged in greater numbers from eggs of M. hapla and M. incognita acrita when placed in tomato root exudates than when placed in distilled water. Through the use of a dialytic membrane to separate the larvae from a tomato seedling, they were able to conclude that accumulation of the larvae of this nematode around germinating tomato seeds is, in part, a response to a dialisable agent or agents emanating from the germinating seed and effective beyond the seedling surface.

Miller and Stoddard (31) reported that nabam

(disodium ethylene bis dithiocarbamate) increased the hatching of Meloidogyne eggs in water solutions; while, in the soil, the material reduced the hatching of these as well as Heterodera eggs.

Marcinowski (29) in 1909 observed that the roots of some plants apparently exude substances which diffuse through the soil, stimulating and attracting their nematode parasites.

The manner in which nematodes respond to root diffusates was thoroughly discussed by Steiner (45). Citing a number of examples, he came to the conclusion that plant parasitic nematodes not only have an ability to recognize host plants, but are able to distinguish the preferred ones. He postulated that the active parts of root exudates are of a rather simple chemical nature, and that the element or elements which stimulate the larvae to hatch from eggs within the cysts of Heterodera schachtii are not the same as those which attract them to the roots.

CHAPTER II

METHODS AND MATERIALS

Pasture and Cover Crop Variety Tests

Test No. 1

For the purposes of this experiment, 48 tar-coated metal culverts, 2 feet in length and 18 inches in diameter, were placed on end in the ground to a depth of 21 inches, leaving one end of each culvert exposed (Fig. 1). The culverts were then filled with virgin Immokalee fine sand. On January 13, 1959, a root-knot nematode (Meloidogyne incognita acrita Chitwood, 1949) was established in the soil by transplanting one heavily infected, mature pepper plant into each culvert plot. The pepper plants were allowed to grow until January 29, when they were cut off at the soil surface. The design of the experiment was a randomized block, with each treatment replicated six times. The following treatments were established:



Fig. 1--Pasture and cover crop variety tests. Culvert plots (top) and buried pots (bottom).

<u>Common Name</u>	<u>Scientific Name</u>
1. Clean fallow	
2. Pangolagrass	<u>Digitaria decumbens</u>
3. Coastal Bermudagrass	<u>Cynodon dactylon</u>
4. Pangolagrass + white clover	<u>Digitaria decumbens</u> + <u>Trifolium repens</u>
5. White clover	<u>Trifolium repens</u>
6. Pensacola Bahiagrass	<u>Paspalum notatum</u>
7. Okra ¹	<u>Abelmoschus esculentus</u>
8. Carpetgrass	<u>Axonopus affinis</u>

Soil samples were taken with a one-inch-diameter soil tube to a depth of six inches in each plot four, six, and eight months after the treatments were established. Each of these samples was placed in a ten-ounce, waxed paper cup and seeded to cucumber used as an indicator plant (Fig. 2). Each cup was drenched at the time of planting with chloranil solution to prevent "damping-off." About two and one-half weeks after the cucumbers were seeded, the roots were washed to remove adhering soil particles and then placed in a beaker of water so that they could be more easily rated for root-knot galling. The rating system employed was: 0 = no evidence of galling, 1 = very slight galling, 2 = slight to moderate galling, 3 = moderate galling, 4 = severe galling, and 5 = very severe galling. These ratings were based on visual observations and were recorded as averages of the ratings made by two people.

¹Okra was replanted several times, apparently because of heavy parasitism by the nematodes.



Fig. 2--Cucumber indicator plants growing in ten-ounce paper cups for root-knot ratings.

Test No. 2

Forty-eight two-gallon glazed pots were placed in two rows and buried in the soil so that only the top inch of each was exposed (Fig. 1). An inch of small gravel was placed in the bottom of each pot and a handful of gravel was also placed outside of the pot at the drain hole to facilitate drainage. The pots were filled about two-thirds full with virgin Immokalee fine sand on which a quart of soil containing the galled roots of cucumber plants was placed; another quart of the virgin soil then was placed on top.

The following treatments were established on November 10, 1959:

<u>Common Name</u>	<u>Scientific Name</u>
1. Crabgrass	<u>Digitaria sanguinalis</u>
2. Pangolagrass	<u>Digitaria decumbens</u>
3. Carpetgrass	<u>Axonopus affinis</u>
4. Pensacola Bahiagrass	<u>Paspalum notatum</u>
5. La. S-1 white clover (Virgin soil)	<u>Trifolium repens</u>
6. La. S-1 white clover (Infested soil)	<u>Trifolium repens</u>
7. Water sedge	<u>Fimbristylis autumnalis</u>
8. Common Bermudagrass	<u>Cynodon dactylon</u>
9. Coastal Bermudagrass	<u>Cynodon dactylon</u>
10. Clean fallow	
11. Flooded	
12. Gahi #1 pearl millet	<u>Pennisetum glaucum</u>

The experimental design was a randomized block with four replications. Soil samples were collected, placed in paper cups, and seeded to cucumber indicator plants as in the previous test at the end of 4, 8, 12, and 16 weeks. Ratings, as previously described, were made two and one-half weeks after the cucumbers were seeded. At the end of the test, the above-ground portions of the white clover plants in Treatments 5 and 6 were harvested, and the fresh weight recorded.

Laboratory and Greenhouse Tests

Pangolagrass extract tests

Ten-gram samples of Pangolagrass roots, stems, or leaves were mascerated in a food blender for 30 seconds in 100 ml. of tap water. These mascerated materials were filtered and stored in a refrigerator at a temperature of about 40°F. until used.

The effect of these extracts on M. incognita acrita was studied by applying aliquots of the extracts to cucumber plants seeded in soil infested by this nematode. Tap water was applied to the check pots. In each of the six experiments that were conducted with these extracts, the treatments were arranged in a randomized block design

and replicated six times. The treatments were applied daily. About three weeks after the tests were initiated, the soil was washed from the cucumber roots so that they could be rated for root-knot galling.

In the first two experiments, extracts from Pangolagrass roots were used, and no effort was made to differentiate between the extract obtained from young Pangolagrass roots and those obtained from the older roots. However, in all later tests the extracts of the old and the young roots were considered separately so that any difference in the effects of the two types of roots could be studied. In the third test, four different extracts--from young and old roots, stems, and leaves--were applied. In the three subsequent tests, only the extracts from the young and the old roots were used.

Egg-hatching test

Egg masses of M. incognita acrita were collected from infected cucumber plants and placed in Syracuse dishes. Eight egg masses were placed in each of eighteen Syracuse dishes. These were divided into three treatments replicated six times.

The extracts were collected and prepared as described in the previous test and were applied at the rate of 4 ml.

per dish. At the end of two weeks, the hatched larvae were counted, and the unhatched eggs were removed to freshly prepared extracts or water. The larvae were counted again two weeks later, and the total number of emerged larvae was recorded.

Root diffusate test

Eighteen one-gallon glazed pots were filled with virgin Immokalee fine sand. Individual sprigs of Pangolagrass were used to plant nine pots, and Pangolagrass sod was planted in the other nine. A glass tube and rubber stopper were placed in the side drain hole of each pot. The pots were watered daily with an excessive amount of water, and the leachates from the pots were collected for use in this test.

Thirty-six standard eight-inch red clay greenhouse pots were filled with root-knot nematode (M. incognita acrita) infested soil. Eighteen of the pots were seeded to Louisiana S-1 white clover, and the other 18 were seeded to tomatoes. The white clover had been inoculated with the correct nitrogen-fixing bacteria, and the tomatoes and clover were fertilized to produce optimum growth of the plants.

The pots of tomato and clover were each divided into three groups which received the treatments listed below:

Louisiana S-1 white clover
Old root leachate
Young root leachate
Tap water check

Tomato
Old root leachate
Young root leachate
Tap water check

The pots were arranged on a greenhouse bench in a randomized block design with six replicates of each treatment.

Soil samples were taken from each pot with a cork borer at the end of 2, 4, 6, 8, and 12 weeks and, as in the other tests, were seeded to cucumber indicator plants. Roots of the cucumbers were rated three weeks later for root-knot galling.

Field Tests

Ft. Pierce Test No. 1

A one-acre block of Immokalee fine sand at the Indian River Field Laboratory (block 1-E) of the Florida Agricultural Experiment Station was used for this test. It had been planted to tomatoes in the spring of 1956 and, after the final harvest, the tomatoes were disked and the land was leveled. The block was divided into nine plots, each measuring 24 feet wide and 250 feet long, with ten-foot alleyways between the plots. Three different soil

management practices were used, with each being replicated three times. These were: (1) planting the land to Pangolagrass by scattering sprigs and disking them into the soil, (2) maintaining the land in a clean fallow condition with frequent disking, and (3) allowing volunteer weeds and grasses to become established with no cultivation. Soil samples were taken periodically for nematode analysis. The total plant parasitic nematode population, other than root-knot, was determined by the Christie-Perry method (13), and root-knot incidence was determined by seeding cucumber indicator plants and observing their roots.

In the spring of 1959, or about two and one-half years after the above cultural practices were started, the north half of each plot was disked, bedded, and planted to tomatoes. In the fall of the same year, the south half of each plot was planted to tomatoes. The tomato plants were transplanted from nematode-free seed benches directly to the field. Normal cultural practices were employed to assure optimum growth of the tomatoes. Soil samples were obtained periodically while the tomatoes were growing. Root-knot nematode population levels were estimated as previously described, and examinations of roots were made for the possible presence of other plant nematodes.

Ft. Pierce Test No. 2

Another field test was initiated in order to study the effect of mixed stands of Pangolagrass and either white clover or crabgrass compared with a relatively pure stand of Pangolagrass. Two areas at the Indian River Field Laboratory were selected for the test. In one area, the plant cover consisted of about 40 per cent Pangolagrass, 25 per cent sedges, and 35 per cent white clover, while in the other area the plant cover consisted of about 50 per cent Pangolagrass and 50 per cent crabgrass. Ten plots, each ten feet square, were marked off in each area and four treatments of five replicates each were applied. One treatment in each area was designed to stimulate the growth of a pure stand of Pangolagrass, while the other treatment was designed to favor the mixed plant growth. The following treatments used in this test were applied in October, 1959, and May, 1960.

Pangolagrass, sedges, white clover plots

1. 300 pounds ammonium sulfate per acre
2. 500 pounds 0-8-24 fertilizer per acre, and the plants were mowed

Pangolagrass, crabgrass plots

1. 100 pounds 9-6-6 fertilizer per acre, and the plants were mowed
2. No fertilizer

Periodically, soil samples were taken in order to study the effects of the treatments on the nematode populations. The M. incognita acrita populations were determined with the aid of cucumber indicator plants as previously described. Other plant parasitic nematodes were removed from the soil by the Christie-Perry (13) method and actual counts of the number of specimens per 100 ml. of soil were made.

Belle Glade test

This test was conducted on Everglades peaty muck soil at the Everglades Experiment Station at Belle Glade, Florida. It included three treatments replicated five times in a randomized block design. The plots were 20 feet wide and 25 feet long with 20-foot alleyways. A celery experiment had been conducted in this block during the fall of 1959, and the area was heavily infested by root-knot nematodes (M. incognita acrita and M. arenaria). The soil was disked following the celery harvest in order to incorporate the celery residue, and the treatments were established on February 10, 1960. The treatments were: (1) sprig planting of Pangolagrass, (2) clean fallow maintained by frequent disking, and (3) native weeds and grasses were allowed to become established. The Pangola-

grass plots were mowed frequently in order to control the weed growth.

Soil samples were taken every two months and seeded to cucumber indicator plants to determine the root-knot populations. Every four months during the test soil samples were taken and the Christie-Perry technique was used in determining the presence of all other plant parasitic nematodes.

Gainesville test

A block of Arredondo fine sandy soil at the Entomology Unit of the Florida Agricultural Experiment Station at Gainesville, Florida, was used for this test. The land had been planted previously to white sweetclover, and the M. incognita acrita and sting nematode (Belonolaimus longicaudatus Rau, 1958) populations were at a moderate level when the test was initiated. The plot area was thoroughly disked on April 15, 1959, and the test crops were planted on May 4, 1959. The crops used were Pangolagrass, Coastal Bermudagrass, and cowpeas. The cowpeas were followed by native weeds and grasses during the fall of 1959. Coastal Bermudagrass and Pangolagrass were established by scattering sprigs of the grasses evenly over the plots and pushing them into the soil with the blade of a shovel. Cowpeas

were planted by broadcasting the seed and covering them lightly with soil. Each treatment was replicated four times. The alleyways were disked frequently to keep them free of weeds. Fertilizer was applied, and the plots were irrigated with overhead sprinklers. Soil samples were taken periodically and seeded to cucumber indicator plants for the purpose of obtaining root-knot data. Also, data on total nematode populations were obtained from each plot every four months during the course of the experiment.

Commercial farm survey

A survey was conducted to determine the distribution of M. incognita acrita in an 80-acre tomato field in Delray Beach, Florida. Half of the field had been planted in tomatoes for four years and the other half had been maintained in a tomato-Pangolagrass rotation program. The rotation consisted of one year in tomatoes, two years in Pangolagrass, and tomatoes during the fourth year. The two fields were separated by a large irrigation ditch. After the last harvest more than 1,200 tomato plants, selected at predetermined intervals in the two fields, were lifted from the soil and their roots rated from 0-4 for root-knot galling.

CHAPTER III

RESULTS AND DISCUSSION

Pasture and Cover Crop Variety Tests

Test No. 1

Data obtained from the cucumbers indicated a wide variation in the effects of the various treatments on population levels of M. incognita acrita (Table 1). Clean fallow and Coastal Bermudagrass apparently reduced the populations of M. incognita acrita to an extremely low level within four months after the treatments were established and there was no evidence that they survived Pangolagrass treatment. Pensacola Bahiagrass supported a moderate root-knot population, while Common Bermudagrass and okra supported massive populations. Louisiana S-1 white clover became established slowly and had allowed a large population of the parasite to build up within four months after being planted. The same occurred when Louisiana S-1 white clover was planted in combination with Pangolagrass (Fig. 3).

Results obtained from the six-month sampling indi-

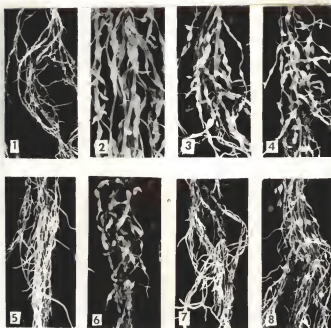


Fig. 3--Cucumber roots showing the effect of several pasture and cover crops on the cotton root-knot nematode population four months after the treatments were initiated.

(1) Pangolagrass, (2) white clover, (3) white clover plus Pangolagrass, (4) Pensacola Bahiagrass, (5) Coastal Bermudagrass, (6) carpetgrass, (7) clean fallow, (8) okra.

cated that nematode populations were reduced by Pangolagrass plus white clover, while the white clover grown alone had died, probably as a result of severe nematode infection. Nematodes were found in only one replicate of Pangolagrass, possibly the result of contamination in sampling the treatments. Root-knot had been reduced to a very low level by Coastal Bermudagrass and was eliminated in the clean fallow plots. The nematode population on okra fluctuated considerably, due probably to the frequent replanting that was necessitated by death of the plants. Common Bermudagrass and Pensacola Bahiagrass plots all contained high populations of M. incognita acrita after six months.

After eight months root-knot was eliminated from both the Pangolagrass and the clean fallow plots, and was reduced to a very low level in the Coastal Bermudagrass plots. Pangolagrass in association with white clover had reduced the population to a lower level than was present in the six month's sampling. The white clover did not survive and therefore was not sampled. Pensacola Bahiagrass, Common Bermudagrass and okra had each maintained the nematode population at a high level throughout the test.

Test No. 2

Root-knot was apparently eliminated within eight weeks from the Pangolagrass and clean fallow plots (Table 2). Flooding reduced the population to a very low level within eight weeks, but twelve weeks were required for eradication.

Crabgrass, Pensacola Bahiagrass, and Common Bermudagrass supported moderate population levels throughout the test. Crabgrass and Common Bermudagrass are weeds found in most of the vegetable fields of South Florida, and Pensacola Bahiagrass is frequently utilized in rotation programs.

Nematode populations built up and remained at high levels on carpetgrass, Gahi millet, sedge, and white clover. "Damping-off" disease of the indicator plants was more severe in the white clover and sedge treatments, but the high incidence of the disease in these treatments apparently was not associated with the high nematode populations since other treatments had equally high nematode populations and little "damping-off."

White clover grown in nematode-free soil showed no root-knot galling in any of the samples, suggesting that contamination had been held to a minimum. The yield from clover grown in nematode-free soil was greater than from

clover grown in association with M. incognita acrita (Table 3).

It is apparent from this test that the population of M. incognita acrita may increase when associated with some of the weeds and grasses previously thought to be immune to attack by this nematode. The data suggest that crabgrass, carpetgrass, white clover, and the sedge should be eliminated from rotation programs. They also confirmed the results of Burton et al. (7), who found that populations of M. incognita acrita increase in the presence of plantings of Bermudagrass and Pensacola Bahiagrass.

The results obtained from this test were most interesting from several aspects. They indicate that certain grasses may be extremely useful in controlling at least some plant-parasitic nematodes. Thus a series of experiments was planned to investigate the situation in more detail.

Laboratory and Greenhouse Tests

Pangolagrass extract tests

In the first test of this series, there was no galling on the six cucumber plants growing in soil infested by M. incognita acrita which received water extracts of

Pangolagrass roots. Those plants which received tap water, on the other hand, were severely galled. When this test was repeated under similar conditions, both the treated and the untreated plants were galled.

Further tests were conducted to determine the reasons for these conflicting data. In the third test, extracts of the various parts of the Pangolagrass plant were tested against M. incognita acrita (Table 4). The extracts from the leaves and stems were not effective in reducing populations of the parasite. The galling on the roots of plants receiving the extract from young white roots was more severe than on check plants, while the extract from the old roots apparently prevented root-knot development.

Further tests comparing extracts obtained from the young roots, extracts from the old roots, and a tap water check produced similar results (Table 5). Thus, by virtue of the increase in galling on the plants receiving the young root extract, such extract might have stimulated a larger percentage of the eggs to hatch. The old root extracts apparently prevented, by some means, the larvae from entering the roots of cucumber.

The data strongly indicated direct physiological effects of Pangolagrass root extracts upon at least the

early larval stages of M. incognita acrita. Quite possibly the egg also might be affected during embryonic development. The first larval stage of this nematode is passed while within the egg shell, and the larvae emerge or "hatch" as second stage, or infective larvae. Very little is known of the processes by which nematode larvae are stimulated to emerge from the eggs. In the case of the root-knot nematodes, the larvae apparently emerge rather slowly, but observations during the course of this work indicate that emergence may sometimes be accelerated.

The data suggest that the immature roots of Pangolagrass produce a material which stimulates development of the early larval stages of this nematode, and, in addition, that the more mature roots of this plant produce materials which actually kill the larvae after they emerge into the soil.

Hatching test

When the extracts of immature Pangolagrass roots were applied to the egg masses of M. incognita acrita, the larvae emerged in high numbers (Table 6), while the extract of the old roots apparently prevented hatching of most of the eggs. The larvae that emerged after application of the old root extracts were sluggish and lived generally for less

than two days in the dishes. When the root extract was removed and replaced with tap water, few additional larvae emerged. Thus it is suggested that a toxic material is present in the roots of old Pangolagrass which directly inactivates larvae within the egg shell.

The results of this test somewhat clarify the previously obtained conflicting data. If the root systems selected for the earlier tests consisted primarily of young roots, then more severe galling would be expected than in a tap-water-treated plant. However, if the extracts were made from the old roots, then few if any galls would be expected.

Further tests were indicated at this point to determine if these root extracts are exuded into and leach through the soil.

Leachate test

When the leachate from newly planted Pangolagrass sprigs was applied to white clover and tomato seeded in nematode-infested soil, the indicator plants were more severely galled after four weeks (Table 7) than they were for the tap water check. However, the nematode rating for those plants receiving the leachate decreased sharply by the sixth week, and by the tenth week, there was no

further evidence of galling on the indicator plants.

The leachate from sod-planted Pangolagrass appeared to reduce the galling on the indicator plants very rapidly and, by the sixth week, all evidence of galling on the indicator plants had been eliminated. In comparison, the tap water check allowed the nematode populations to increase to a high level and, at the end of the test, the clover yield was lower in this treatment than that from the other two treatments (Table 8).

In these laboratory and greenhouse tests, it became obvious that Pangolagrass roots contain at least two biologically active materials which have important effects on M. incognita acrita. Hatching of the eggs of this nematode is apparently stimulated by one of these materials, while the second material apparently is toxic to larvae of the nematode.

Thus a most interesting situation apparently exists whereby a nematicidal material is produced by a plant after another chemical product of this plant has stimulated the nematode to develop to a stage which is most susceptible to toxins. The nature of these compounds has not been investigated due to the complexity of such investigations. If they could be identified and synthesized, they might prove useful as chemical nematocides.

At this stage during the investigation, the question arose as to field application. In other words, would Pangolagrass reduce nematode populations under field conditions? Also, would the grass need to be cultured so as to eliminate native hosts for the nematodes? Under field conditions, several species of plant nematodes commonly occur together. All tests to this point had been concerned with only the one species, and no conclusions could be drawn as to possible effects on other genera or species.

Ft. Pierce Test No. 1

When the plots were disked and bedded in preparation for the tomato crop, the soil in the Pangolagrass and in the weed and grass plots was in much better condition for planting than the soil in the clean fallow plots. The root systems that were left in the soil after the Pangolagrass and weed treatments effectively reduced erosion due to high wind and heavy rain. The soil in the clean fallow plots eroded severely under the same conditions (Fig. 4). The tomato plants growing in the clean fallow plots also were damaged by the exposure of their root systems to the drying effects of the sun and wind.

Examination of the tomatoes planted in the spring of 1959 (Table 9), indicated that the culture of Pangola-



Fig. 4--The effect of Pangolagrass sod on tomato bed erosion from heavy rains. (Top) Soil previously maintained in clean fallow condition. (Bottom) Soil previously in Pangolagrass. Note lack of erosion in this bed.

grass was at least as effective as clean fallow in reducing populations of M. incognita acrita. Roots of tomato plants grown after Pangolagrass or clean fallow showed no evidence of nematode injury. The populations of this nematode had been maintained at a relatively high level by the weed and grass treatments (73 per cent of the tomato plants were infected).

In the fall test, the Pangolagrass was more effective than clean fallow in reducing the nematode population. Sixteen per cent of the tomatoes from the clean fallow plots were infected, while only 3 per cent from the Pangolagrass plots were infected. Eighty per cent of the tomato plants growing in the weed and grass plots were infected with M. incognita acrita.

Two conclusions could be drawn from the results of this test. First, the production of Pangolagrass for a period of two and one-half years virtually eliminated M. incognita acrita from this type of soil under field conditions. Secondly, the culture of Pangolagrass appeared vastly superior to clean fallow for soil conservation purposes.

Ft. Pierce Test No. 2

The first test of this series resulted in a favorable response of white clover to the 500-pound-per-acre rate of 0-8-24 fertilizer, while Pangolagrass effectively eliminated the white clover in those plots receiving 300 pounds of ammonium sulfate per acre. In the spring of 1960, Pangolagrass made up more than 90 per cent of the plant cover in those plots receiving ammonium sulfate. The nematode population (Table 10), also was reduced rapidly by this treatment, and one year after initiation of the treatments no evidence of the nematode could be found in the Pangolagrass treatment. As a result of cool weather which predominated during this test, crabgrass was reduced in all plots. Rapid growth of the Pangolagrass receiving fertilizer apparently prevented re-establishment of crabgrass the following spring. Crabgrass did re-establish rapidly in those plots which were not fertilized; and in the spring, made up about one-half of the plant cover. At the end of the test, M. incognita acrita apparently had been eliminated by the nearly pure stand of Pangolagrass induced by the high fertilization rate. Populations of the root-knot nematodes remained high in plots that received no fertilizer. These results indicate that Pangolagrass must predominate for

effective nematode control, and that proper fertilization favors the Pangolagrass in competition with the native flora.

Belle Glade test

The response of M. incoqnita acrita to Pangolagrass was slower than the response to clean fallow on the organic soil. The root-knot nematode population survived in the Pangolagrass plots for 14 months (Table 10), while only 8 months were required to eliminate them in the clean fallow plots. This delay may have been due, in part, to the heavy weed population which infested the Pangolagrass plots soon after the experiment was begun. Weeds were not allowed to become established in the clean fallow plots. The plots in which native weeds and grasses were allowed to become established supported a high nematode population throughout the test.

Populations of stubby-root nematode, T. christiei, were low in the test area and after 18 months they were eliminated in the Pangolagrass and clean fallow plots (Table 11). The populations of this nematode increased to a higher level in the native weed and grass plots.

The spiral nematode, Helicotylenchus nannus, population remained at about the same level throughout the test

in Pangolagrass (Table 12) and it was eliminated by the clean fallow treatment in 18 months. In association with the native weeds and grasses, the population built up to a higher level.

Gainesville test

In this test, Pangolagrass and Coastal Bermudagrass became established very slowly, and early in the test weed populations were at a high level in both treatments. Within four months after the treatments were initiated, the weeds were almost eliminated by frequent mowing and heavy applications of fertilizer. The nematode populations remained at high levels during this period. Populations of M. incognita acrita were reduced to a trace in about 12 months (Table 13) and were eliminated in less than 16 months by Pangolagrass. Coastal Bermudagrass was not as effective, and a trace of galling due to this Nematode was present after 16 months. Belonolaimus longicaudatus populations increased in the presence of Pangolagrass and were at a high level at the end of the test (Table 14). Populations of B. longicaudatus remained at a moderate level on both cowpeas and Coastal Bermudagrass. Ring nematodes (Criconemoides) populations increased in the presence of all three treatments (Table 15).

The results of this test are important for two

reasons. First, the reduction in numbers of M. incognita acrita by the two grasses confirmed previous results obtained at Ft. Pierce. The soil type, environment, and nematode populations differ at Gainesville from those at Ft. Pierce. Second, these data plus other observations indicate that both Pangolagrass and Coastal Bermudagrass are hosts for the sting nematode. In all probability parasitism by the sting nematode prevented proper establishment of the grasses in this test. It would be interesting to speculate as to why exudates from the roots of these grasses are toxic to a root-knot nematode and yet do not affect the sting nematode. Early larval stages of both species are free in the soil, and thus both must be exposed to the root exudates. Certainly caution must be observed when formulating recommendations for the use of these grasses as control measures for the root-knot nematode.

Commercial farm survey

Data obtained from a commercial farm indicated that a tomato-Pangolagrass rotation program will give effective control of M. incognita acrita. Only 13 per cent of the tomatoes taken from the field maintained in the rotation program were injured by root-knot nematode and the galling was not severe on any plants. In comparison, about 39 per

cent of the tomato plants from the adjoining field which had been in tomatoes for four years were found severely galled.

The nematode populations were most severe near irrigation ditches in both fields. This probably was due to severe root-knot infection of weeds growing along these ditches. When the ditches were cleaned prior to planting, soil was scattered over the field, thus distributing the nematodes which had survived on the weeds.

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APPENDIX

TABLE 1.--Meloidogyne incognita acrita gall ratings on cucumber indicator plants as influenced by pasture and cover crop varieties in Test No. 1

	Root-knot Indices ^a		
	4 Months	6 Months	8 Months
1. Pangolagrass	0.0	0.3	0.0
2. La. S-1 white clover	5.0	. .	. .
3. Pangolagrass + La. S-1 white clover	4.8	3.2	1.5
4. Pensacola Bahiagrass	3.8	3.5	4.3
5. Coastal Bermudagrass	1.3	0.2	0.2
6. Common Bermudagrass	4.8	5.0	5.0
7. Clean fallow	0.2	0.0	0.0
8. Okra	4.5	2.2	4.5

^aEach figure is the average of six replicates rated from 0-5 for root-knot galling.

TABLE 2.--*Meloidogyne incognita acrita* gall ratings on cucumber indicator plants as influenced by pasture and cover crop varieties in Test No. 2

	Root-knot Indices ^a					
	No. of weeks after planting					
	0	4	6	8	12	16
1. Crabgrass	2.0	2.3	3.7	2.7	2.8	1.8
2. Pangolagrass	2.0	0.5	0.5	0.0	0.0	0.0
3. Carpetgrass	2.1	2.0	2.5	3.3	4.0	4.0
4. Pensacola Bahigrass	1.7	1.3	3.8	1.5	3.3	2.0
5. La. S-1 white clover ^b	0.0	0.0	0.0	0.0	0.0	0.0
6. La. S-1 white clover	1.8	0.8	1.3	0.3	3.0	3.3
7. Sedge	c	c	c	4.0	4.0	3.5
8. Common Bermudagrass	1.7	1.3	0.0	0.5	2.7	1.8
9. Coastal Bermudagrass	1.8	1.2	0.0	0.8	0.3	0.3
10. Clean fallow	1.8	0.7	1.0	0.0	0.0	0.0
11. Flooding	1.9	0.7	0.5	0.8	0.0	0.0
12. Gahi millet	1.7	1.7	3.7	c	3.5	4.0

^aRatings made on cucumber indicator plants growing in soil samples from each treatment. Each figure is the average of four ratings from 0-5 as previously discussed.

^bLa. S-1 white clover growing in root-knot-free soil.

^cIndicator plants failed to survive.

TABLE 3.--Louisiana S-1 white clover yields in Treatments
5 and 6 in Test No. 2

Treatments	Harvest Dates	
	Aug. 15,1959	Sept. 5,1959
	(gms.)	(gms.)
Virgin soil	4.5 ^a	4.1
Root-knot infested soil	0.7	0.2

^aThe average of four replicates.

TABLE 4.--Meloidogyne incognita acrita gall ratings on cucumber indicator plants as influenced by Pangolagrass plant extracts

Treatment	Root-knot Indices ^a
Mature root extract	0.2
Young root extract	3.4
Leaf extract	2.4
Stem extract	2.8
Tap water	2.6

^aThe average of five replications rated from 0.5 for root-knot galling caused by M. incognita acrita as previously discussed.

TABLE 5.--Meloidogyne incognita acrita gall ratings on cucumber indicator plants as influenced by Pangolagrass root extracts

Treatment	Tests		
	#1	#2	#3
Mature root extract	0.5 ^a	0.0	0.1
Young root extract	1.5	3.2	3.3
Tap water check	1.1	2.3	2.5

^aEach figure is the average rating from six replicates rated from 0-5 for root-knot galling as previously described.

TABLE 6.--The influence of Pangolagrass root extracts on larval emergence from Meloidocvne incognita acrita eggs

Treatment	Larvae Emerged
Mature root extract	54
Young root extract	1,510
Tap water	724

TABLE 7.--Meloidogyne incognita acrita gall rating on cucumber indicator plants in soil from white clover and tomato growing in root-knot infested soil as influenced by Pangolagrass root diffusates

	Root-knot Indices at Biweekly Intervals following Treatments				
	2	4	6	8	10
Clover					
Tap water	2.6 ^a	1.3	2.3	2.6	2.4
Sod leachate	0.6	0.2	0.0	0.0	0.0
Sprig leachate	3.1	3.6	0.9	0.2	0.0
Tomato					
Tap water	2.9	2.1	1.7	2.0	2.1
Sod leachate	0.7	0.4	0.0	0.0	0.0
Sprig leachate	3.4	2.4	1.2	0.5	0.0

^aAn average of six replicates of each treatment.

TABLE 8.--Louisiana S-1 white clover yields in root-knot infested soil as influenced by Pangolagrass root diffusates

Treatment	Yield (gms.)
Tap water	0.1 ^a
Sod leachate	5.9
Sprig leachate	3.6

^aThe average yield from six replications of each treatment.

TABLE 9.--Helicotylenchus nannus populations as influenced by Pangolagrass, clean fallow, and native weeds and grasses in Immokalee fine sandy soil

Treatment	Sampling Dates		
	Oct. 22, 1958	March 23, 1959	June 29, 1959
Pangolagrass	229 ^a	487	104
Clean fallow	0	0	2
Native weeds	9	0	5

^aThe average number of nematodes per 100 ml. soil sample from three replications of each treatment.

TABLE 10.--Meloidogyne incognita acrita populations as influenced by Pangolagrass, clean fallow, and native weeds in an Everglades peaty muck soil at Belle Glade, Florida

Treatment	4-4 1960	6-8 1960	8-1 1960	10-7 1960	12-3 1960	2-5 1961	4-9 1961	6-3 1961	8-1 1961
1. Pangolagrass	3.7 ^a	3.1	2.9	2.3	1.7	0.8	0.0	0.0	0.0
2. Clean fallow	3.4	1.9	0.6	0.0	0.0	0.0	0.0	0.0	0.0
3. Native weeds	3.4	3.2	3.3	3.3	3.1	3.6	3.2	3.5	3.6

^a Each figure is the average root-knot rating (0-4) for cucumber plants growing in samples of soil from each of five replicates of the treatments.

TABLE 11.--Trichodorus christiei Allen populations as influenced by Pangolagrass, clean fallow, and native weeds in an Everglades peaty muck soil at Belle Glade, Florida

Treatment	Sampling Dates			
	June 1960	Oct. 1960	May 1961	Aug. 1961
1. Pangolagrass	7 ^a	4	1	0
2. Clean fallow	5	3	1	0
3. Native weeds	5	12	12	20

^aEach figure is the average of the number of spiral nematodes found in 100 ml. soil samples from each of five replicates of the treatments.

TABLE 12.--Helicotylenchus nannus populations as influenced by Pangolagrass, clean fallow, and native weeds in an Everglades peaty muck soil at Belle Glade, Florida

Treatment	Sampling Dates			
	June 1960	Oct. 1960	May 1961	Aug. 1961
1. Pangolagrass	27 ^a	36	29	24
2. Clean fallow	21	12	3	0
3. Native weeds	24	27	39	33

^aEach figure is the average of the number of spiral nematodes found in 100 ml. samples from each of five replicates of the treatments.

TABLE 13.--*Meloidogyne incognita acrita* gall rating on cucumber indicator plants in Arredondo fine sand planted to Pangolagrass, Coastal Bermudagrass, and cowpea

Cover Crop	Sampling Dates		
	Aug. 15 1960	March 12 1961	July 10 1961
1. Pangolagrass	1.6 ^a	0.4	0.0
2. Coastal Bermudagrass	2.0	1.0	0.1
3. Cowpea	2.8	3.2	2.6

^aEach figure is the average rating (0-5) of root-knot galling on cucumber indicator roots grown in samples of soil from each of five replicates of the treatments.

TABLE 14.--Belonolaimus longicaudatus populations as influenced by Pangolagrass, Coastal Bermudagrass, and cowpea in Arredondo fine sandy soil

Cover Crop	Sampling Dates		
	Aug. 15 1960	March 12 1961	July 10 1961
1. Pangolagrass	32 ^a	38	56
2. Coastal Bermudagrass	38	24	12
3. Cowpea	18	22	20

^aEach figure is the average number of sting nematodes found in 100 ml. of soil taken from each of the five replicates of each treatment.

TABLE 15.--Criconemoides spp. as influenced by Pangolagrass, Coastal Bermudagrass, and cowpea in Arredondo fine sandy soil

Cover Crop	Sampling Dates		
	Aug. 15 1960	March 12 1961	July 10 1961
1. Pangolagrass	9	86	123
2. Coastal Bermudagrass	7	63	119
3. Cowpea	3	50	71

BIOGRAPHICAL SKETCH

James Alwyn Winchester was born August 5, 1927, in Delray Beach, Florida. He attended public schools in Deerfield Beach and Pompano Beach, Florida, and was graduated in 1945 from Levelland High School in Levelland, Texas. He served as a radarman in the United States Navy from 1945 until 1947. He attended the Palm Beach Junior College from 1948 until 1949, when he transferred to the University of Florida. In 1952, he received the Bachelor of Science in Agriculture degree and in 1953 the Master of Science in Agriculture degree, both from the University of Florida.

He did research in pineapple production from 1953 to 1957 and in 1959 he joined the staff of the Indian River Field Laboratory of the University of Florida Agricultural Experiment Stations as Interim Assistant Agronomist. In 1960, he resumed his graduate study, working toward the Doctor of Philosophy degree. In 1961, he joined the staff of the Everglades Experiment Station as Interim Assistant in Nematology.

He is a member of the Soil and Crop Science Society

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He is married to the former Jacqueline Canton and they have three sons, James A., Jr., Jon Canton, and Sterling Ray.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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